Past NHEERL Research on Biological Effects of Mineral and Synthetic Fibers

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NHEERL

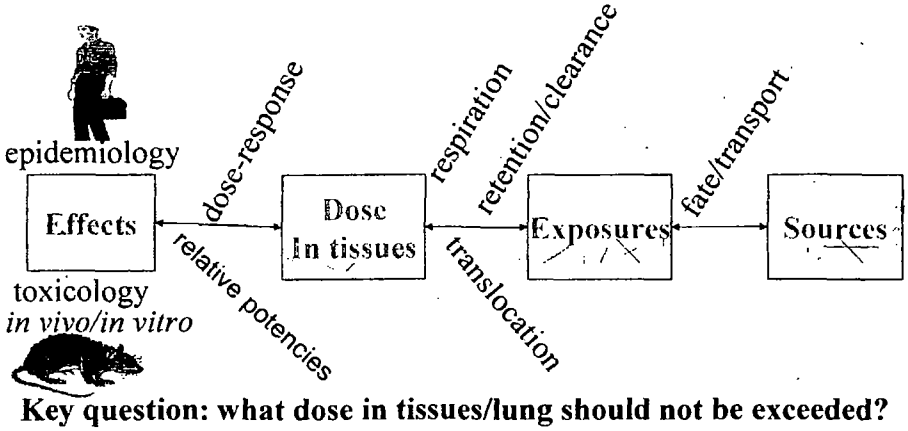
Before NHEERL, ORD researchers (HERL at RTP and NWQL at Duluth) collaborated on investigations of the biological effects of mineral and synthetic fibers that resemble microscopic fibers in dusts generated from asbestos. Animal exposures, pathologies, and in vitro studies were conducted at RTP while characterizations of fiber mineralogies, properties, and exposures were performed at Duluth along with quantitative TEM measurements of doses *in vivo* .

Objectives for this presentation

- **Describe studies, findings, optimized dose metrics, and data available from past ORD research that may be of use for planning toxicity studies involving Libby amphibole fiber samples.**
- **Comment on opportunities for new studies to build on past studies and connect most effectively with toxicity data in the literature.**
- **Mention opportunities for Libby amphibole studies to provide data useful for advancement of fiber dose-response relationships in general.**

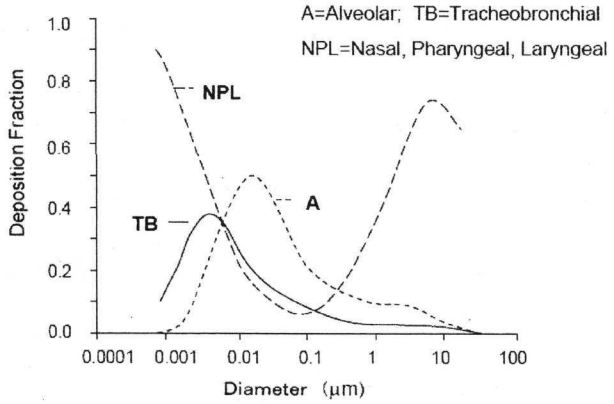
Conceptual Model

for development of criteria to prevent unacceptable health risks from inhalation of complex mixtures of mineral and synthetic fibers



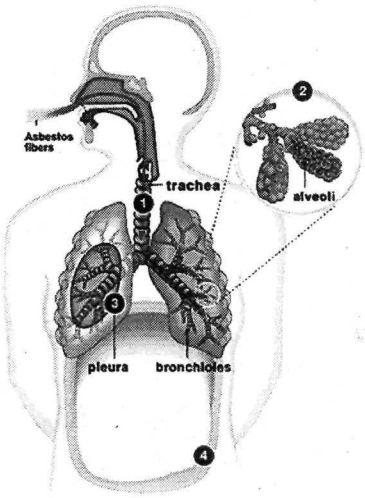
Key question: what dose in tissues/lung should not be exceeded?
Temporal exposure issues - lifetime, short term, early life stages

Deposition of particles in the human respiratory system.

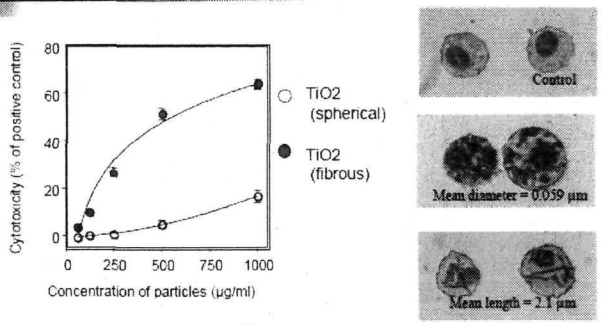


Respiration, translocation, retention/clearance, and dissolution are all kinetically controlled processes that allow some short fibers to reside in alveoli and reach the pleura. Mesothelioma seems to be associated with shorter fibers that reach the pleura.

Insufficient attention has been placed on the influence of exposure duration, intensity, and fluctuations on retained fiber sizes at critical times with respect to disease risks.



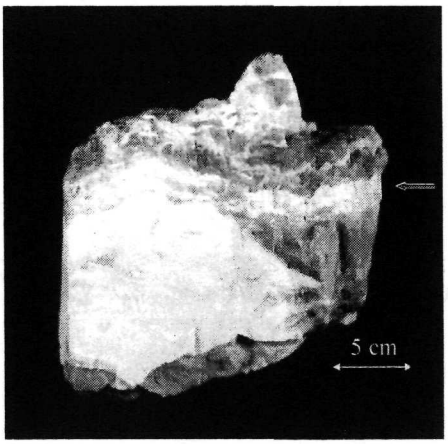
Cytotoxicity of Spherical and Fibrous TiO_2 in rat alveolar macrophages.



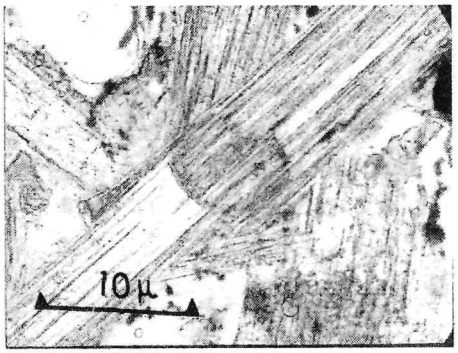
S.Hirano et al., Am. J. Respir. Cell Mol. Biol. 23:313-319 (2000)

Are these potency differences simply related to total surface areas?

Mineral fibers have diverse origins and are often heterogeneously distributed in rocks



Chrysotile asbestos cross-fiber vein

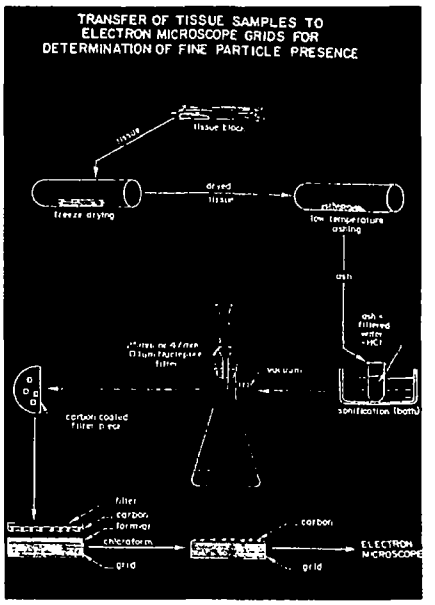


Amphibole crystals in taconite (iron ore) - ferroactinolite replacing hornblende

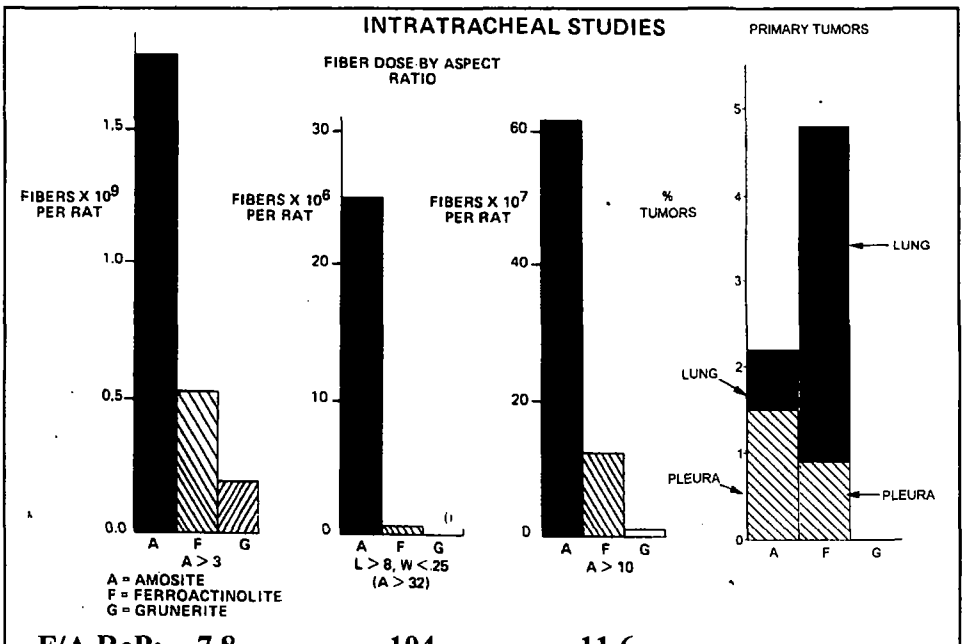
Methods used for analysis of fiber concentrations in water and air samples were adapted for measuring dose-response relationships.

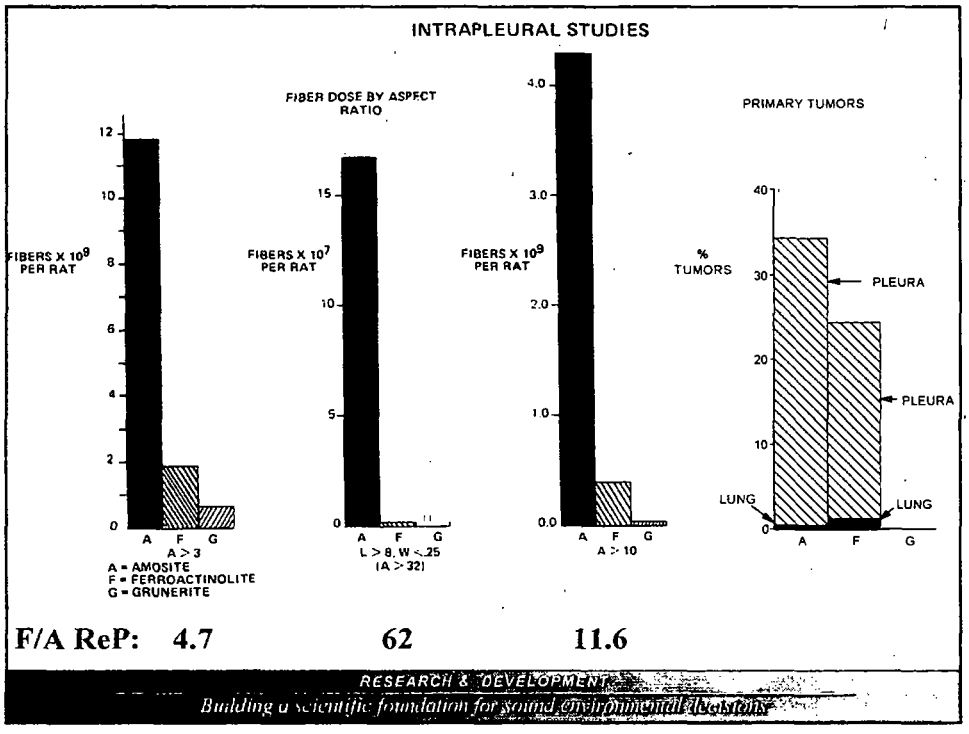
Example: quantitative TEM analyses of whole lung samples from rats over the two year test period provided complete *in vivo* dose characterizations over time.

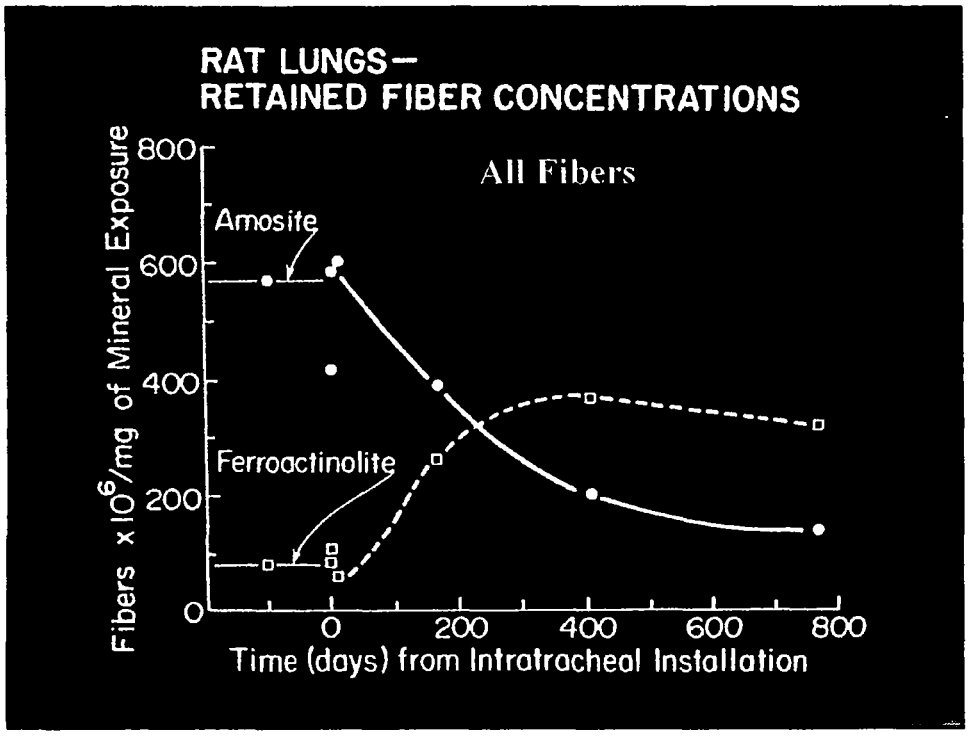
Cook, Coffin, Palekar, *Toxicology Letters, 1982*

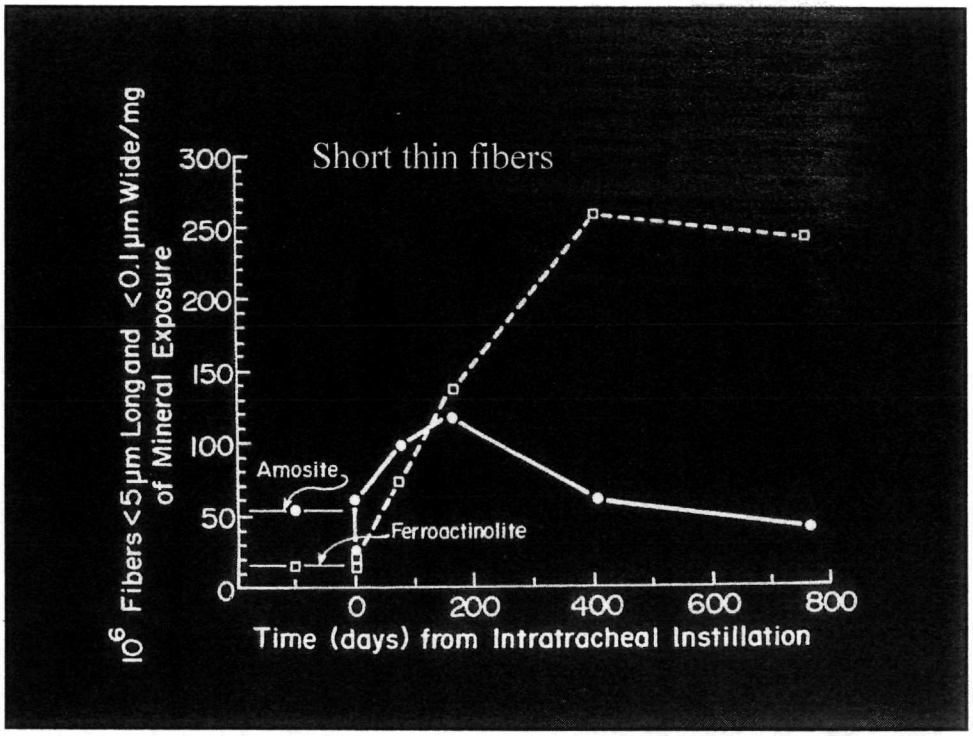


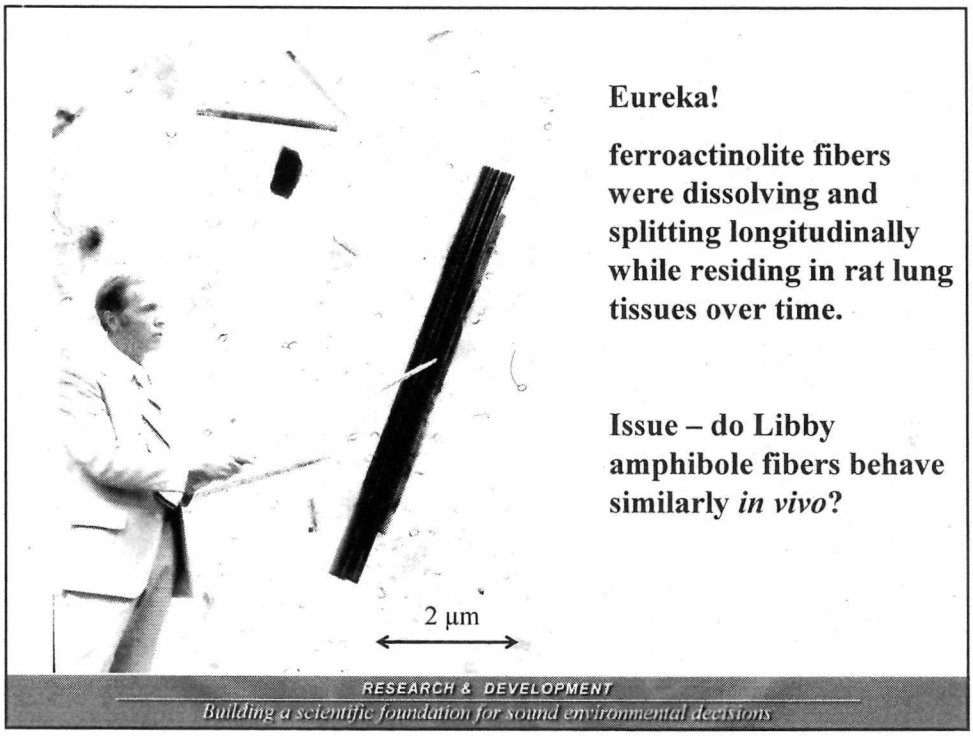
Cook, *Ann. N.Y. Acad. Sci* 1979

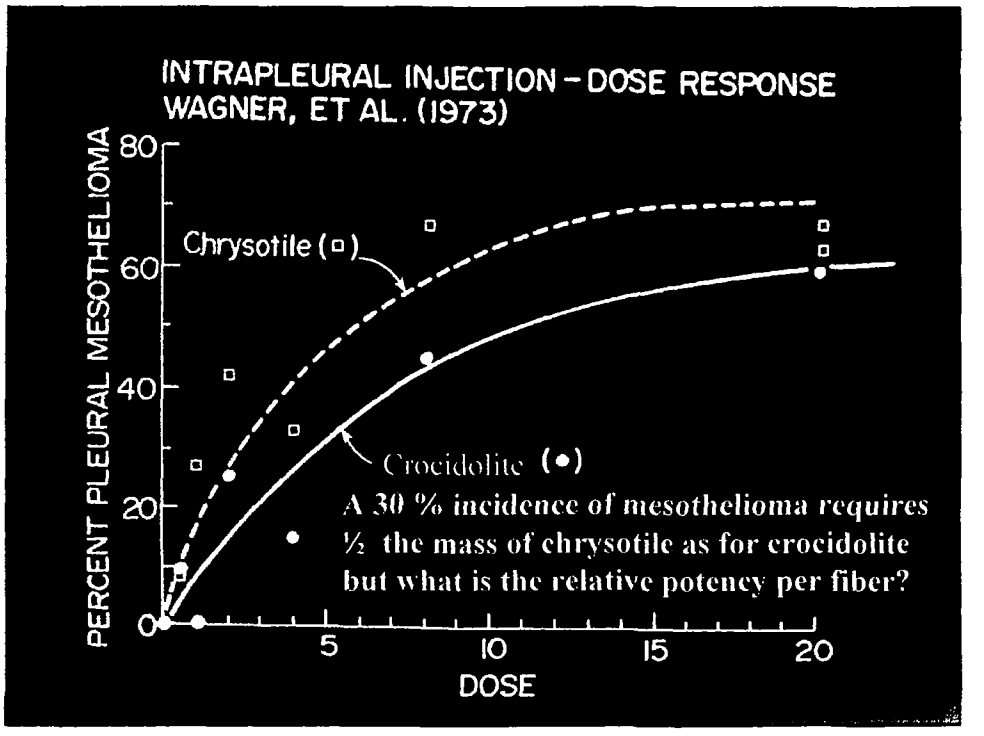


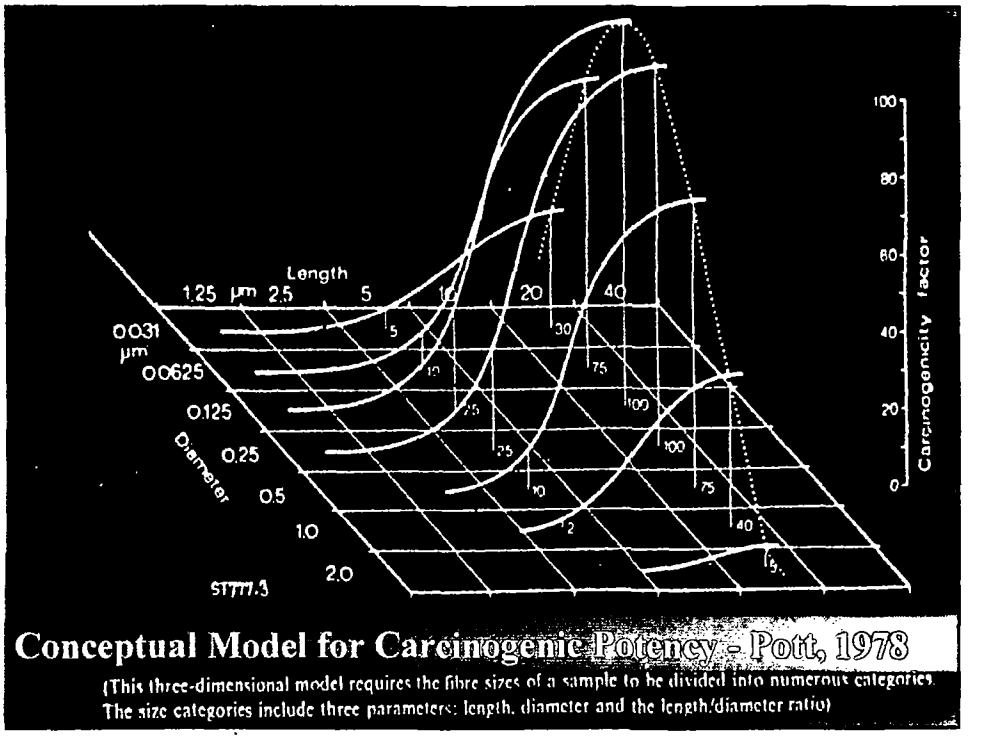

INTRAPLEURAL STUDIES
FIBERS X 10^9 PER RAT
A > 3
A = AMOSITE
F = FERROACTINOLITE
G = GRUNERITE
FIBER DOSE BY ASPECT RATIO
FIBERS X 10^7 PER RAT
L > 8, W < .25
(A > 32)
FIBERS X 10^9 PER RAT
A > 10
PRIMARY TUMORS
% TUMORS
PLEURA
PLEURA
LUNG
LUNG
F/A ReP: 4.7 62 11.6
RESEARCH & DEVELOPMENT
Building a scientific foundation for sound environmental decisions


RAT LUNGS—
RETAINED FIBER CONCENTRATIONS
All Fibers
Amosite
Ferroactinolite
Fibers x 10^6/mg of Mineral Exposure
Time (days) from Intratracheal Installation


Short thin fibers
10^6 Fibers <5 µm Long and <0.1 µm Wide/mg of Mineral Exposure
300
250
200
150
100
50
0
Amosite
Ferroactinolite
0
200
400
600
800
Time (days) from Intratracheal Instillation


2 µm
Eureka!
ferroactinolite fibers were dissolving and splitting longitudinally while residing in rat lung tissues over time.
Issue – do Libby amphibole fibers behave similarly *in vivo*?
RESEARCH & DEVELOPMENT
Building a scientific foundation for sound environmental decisions





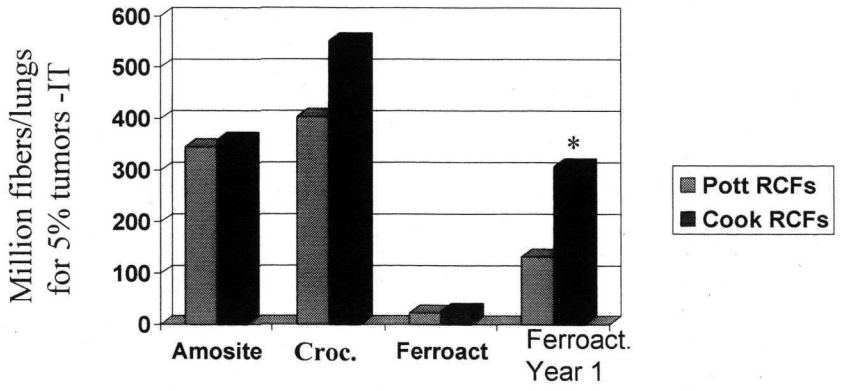
Conceptual Model for Carcinogenic Potency - Pott, 1978

(This three-dimensional model requires the fibre sizes of a sample to be divided into numerous categories. The size categories include three parameters: length, diameter and the length/diameter ratio)

Carcinogenicity Equivalence Dose (CED)

- **Let CED = dose expressed as the number of equivalent reference fibers in the lung or pleura.**
- **CED = $\Sigma(RCF_{i,j})(C_{i,j})$, where $C_{i,j}$ = # fibers/organ or tissue; RCFs are relative carcinogenicity factors; i designates the fiber length/width category and j designates the fiber type.**
- **If amphiboles have equipotent fibers within specified size and shape ranges and a set of proposed RCF values are accurate, CEDs for different amphiboles should be similar for a specific level of effect in a toxicity test.**
- **An initial method for proposing RCFs is to simply determine values that result in agreement of CEDs for different fiber samples in a standard toxicity test.**

Carcinogenicity Equivalence Doses with Alternative RCFs



* For Cook RCFs, Amosite and Crocidolite CEDs at 1 year ≈ Ferroactinolite CED at 1 year.

Archived NHEERL Records Restored

- Large, comprehensive reprint file covering reports published prior to 1985.
- Transmission electron microscope methods development and QA data for a wide variety of samples.
- Fiber dose characterization data base relating to many toxicology studies.

NHEERL DATA BASE CONTENT

- 42 unique mineral and synthetic fiber samples
- 270 individual sample TEM analysis files
- Effects studies represented – EPA, Stanton et al., Wagner et al., Davis et al., others associated with UICC samples
- Number of effective particles measured – 1,186,046
- Values measured for every particle – length, width, SAED/particle identity, shape category (e.g. fiber), area multiplier.
- Additional measurements for selected particles – EDS elemental composition, thickness.
- Sample associated parameters – origin, mass analyzed, filter area, TEM grid area examined, leaching condition, etc.

DATA BASE CONTENT
Basic Types of Samples

- Fiber exposure samples – fibers/mass sample in exposure – IT, IP, Inhalation, IPer, in vitro
- Whole lung samples – fibers/lungs over time
- Extra-pulmonary tissue samples for fiber translocation measurements – fibers/g tissue or /organ over time
- Leached fiber exposure samples to simulate alteration over time in lung and other tissues
- Methods development and QA samples

Potential Data Base Benefits

- High quality data generated by one laboratory.
- Routine calculations of fiber or total particle number, mass, or surface area concentrations for total sample or any set of defined L x W categories.
- Applicability to exposures from many studies – improving, standardizing dose metrics across studies possible.
- Emphasizes tissue burden dose over time.
- Data for simulation of *in vivo* alteration of fiber samples over time.
- Ability to test relative potency values for specified fiber length x width categories.
- Describe fiber size relationships associated with translocation from lung or pleura over time.

Status of NHEERL Dose-Response Data

- Over 270 electronic data files characterizing fiber doses have been restored from archived magnetic tapes and quality assured based on paper records.
- Files have been remodeled to provide an Excel spreadsheet format allowing versatile calculations.
- User guide and documentation drafted.
- Work on initial data interpretation papers and derivation of relative potency factors is beginning.
- Data base should provide more consistent and relevant measures of exposures for a number of important published studies.
- Uncertainties for potencies associated with fiber length/width characteristics, as well as mineral type, may be reduced.
- Data base may be useful for exploring potential risks associated with inhalation of new fine particles provided by nanotechnology.
- QA Review – Feb 22, 2006.

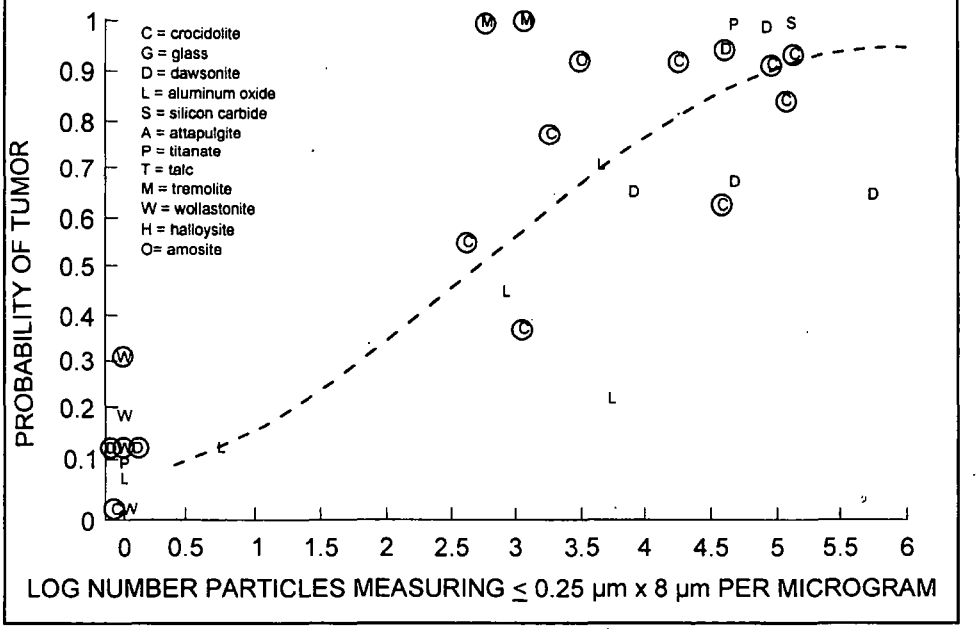
Major Unreported NHEERL Studies

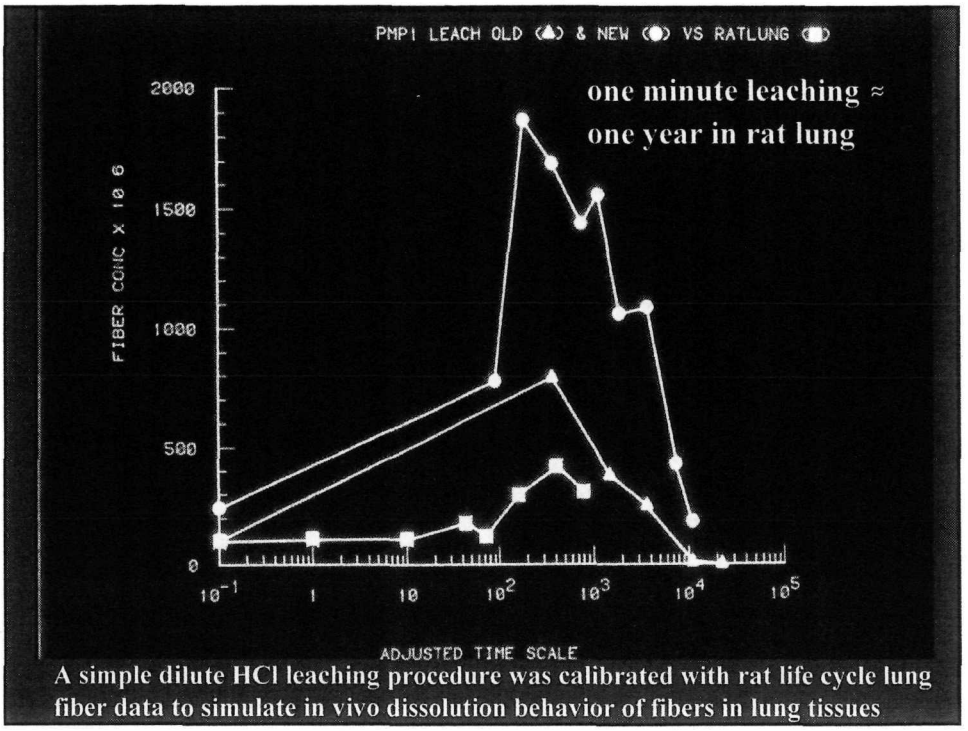
- **Fiber translocation - temporal *in vivo* dose relationships.**
- **Determination of optimum relative carcinogenicity factors based on size, shape, and durability of fibers.**
- **Simulation of fiber dissolution and morphology alteration in lung tissues.**
- **Validate, improve carcinogenicity equivalence dose model with TEM data for *in vivo* dose-response in rats for about 40 fiber types.**
- **Re-analysis of 29 fiber samples from NCI study (Stanton et al. 1981) for improved QSARs to describe pleural sarcoma potency of fibers.**

The Stanton Study and EPA Reanalysis

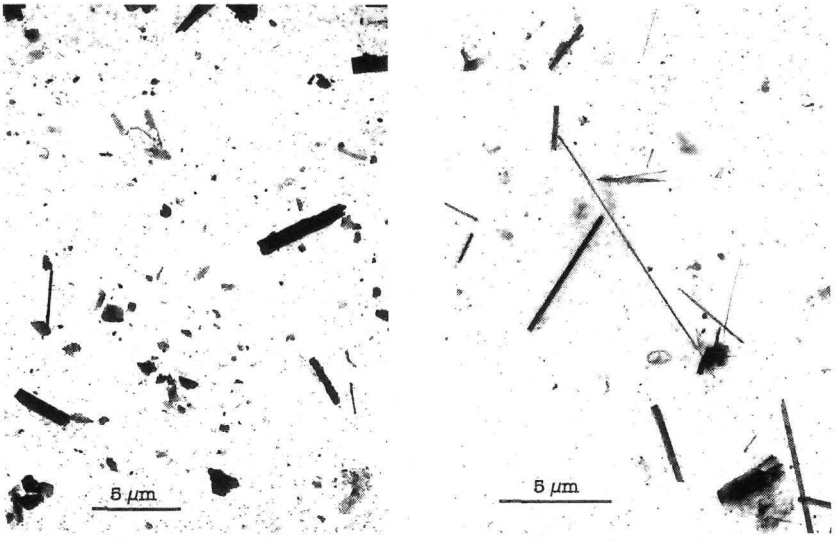
- **Stanton et al. 1981 has frequently been cited as evidence for long fiber potency.**
- **An unconventional approach was used for calculating particle concentrations in rat pleural exposures which were characterized as order of magnitude estimates.**
- **Although Stanton et al. found log # fibers > 8 μm long and ≤ 0.25 μm wide to best correlate with incidence of pleural sarcoma, significant correlations were found for other fiber size categories.**
- **Our preliminary statistical analysis in 1985 using EPA TEM data indicated fiber thinness was the strongest morphological descriptor of potency.**
- **The EPA TEM data for 29 Stanton Samples should allow application of the relative potency model to determine additive contributions of all fiber sizes with consideration of in vivo durability effects on carcinogenicity.**

Stanton et al. Samples Reanalyzed by EPA





A simple dilute HCl leaching procedure was calibrated with rat life cycle lung fiber data to simulate in vivo dissolution behavior of fibers in lung tissues

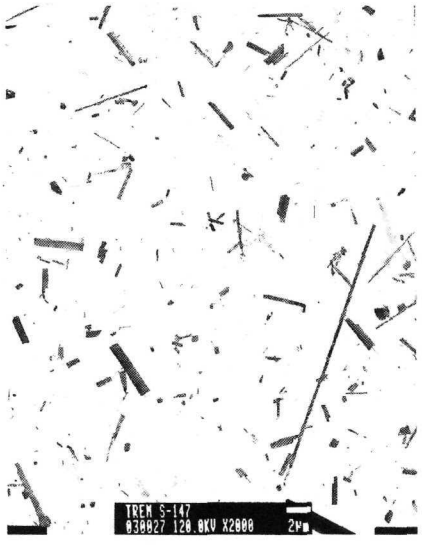


Exposure

One Year in Tissue -Simulation

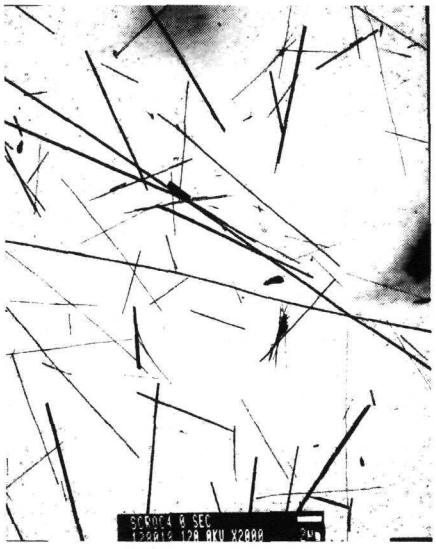
Ferroactinolite – Cook et al. 1982; Coffin et al. 1982

Exposure



10 Years in Tissue - Simulation

Tremolite – Stanton 100% Tumor Probability

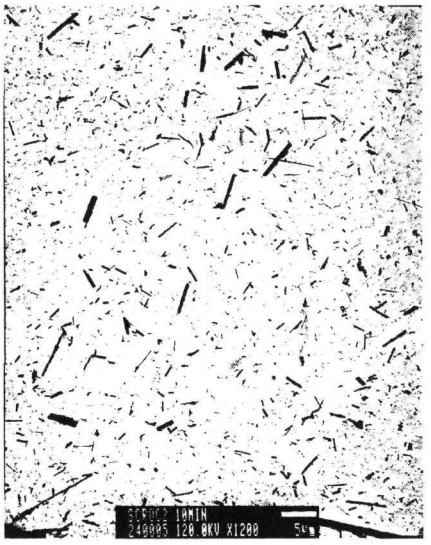


Exposure

60 Years in Tissue Simulation

Crocidolite 4 – Stanton 86% Tumor Probability

Exposure



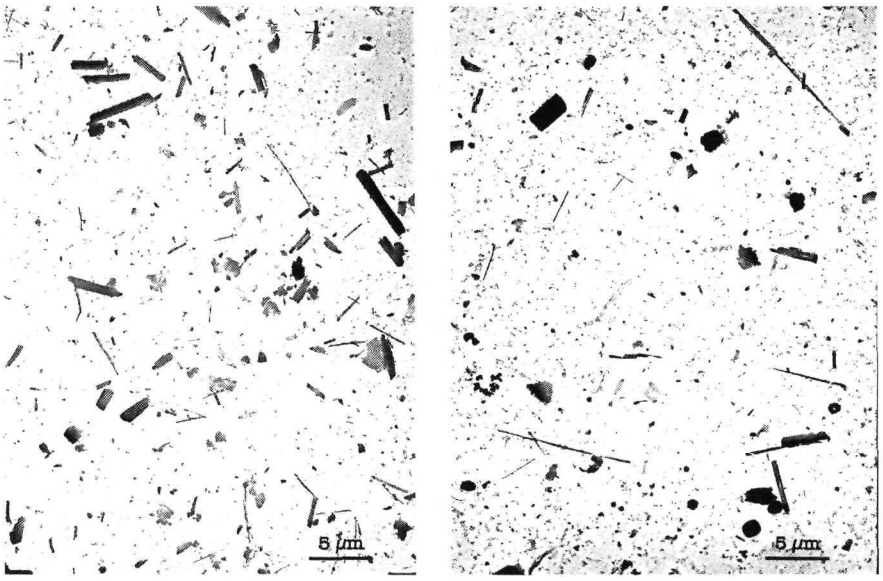
10 Years in Tissue Simulation

Crocidolite 2 – Stanton 93% Tumor Probability

Exposure

2 Months in Tissue Simulation

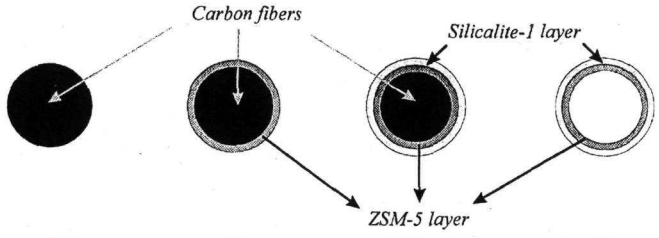
Dawsonite 6 – Stanton 13% Tumor Probability



Exposure 1 Year in Tissue Simulation

Erionite – Rome, Oregon – Wagner et al. 1985; EPA - Coffin et al. 1992

Carbon nanofibers are used to produce zeolite nanotubes



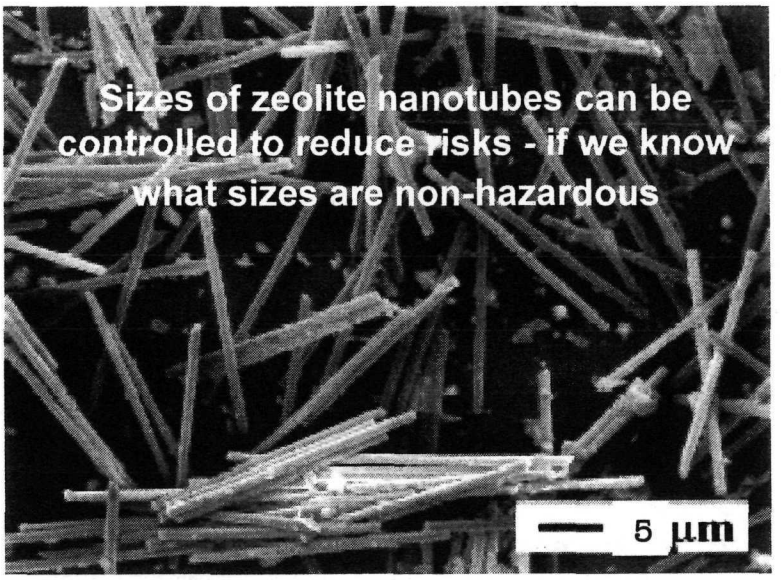
A **Carbon fibers**

B **ZSM-5/fiber composites**

C **Silicalite-1/ZSM-5/fiber composites**

D **Hollow ZSM-5/silicalite-1 tubes**

Note – nano fabrication techniques may be the best approach for creating uniform fiber samples for toxicity testing in the future.



Concluding Comments

- **The *in vivo* potency of Libby Amphibole fibers relative to asbestos fibers for both non-cancer and cancer effects seems to be the central issue.**
- **Designing toxicity studies that will produce dose data comparable to those in the NHEERL data base would seem to be advantageous.**
- **Research on *in vitro* methods for screening relative potencies of diverse mineral and synthetic fiber samples could be aided by use of standard samples already characterized.**
- **Predicting Libby Amphibole *in vivo* durability and potential for morphology alteration can be done using the leaching assay developed by NHEERL.**